content determinations were performed within 2 weeks of the last gas-bombardment cycle. The results of all these studies are summarized in the table.

Although the Kr-surface area measured before the 12-cycle treatment appears to be slightly greater than that measured after treatment, the values fall within expected experimental error and little more can be said on this issue. The last column in the table indicates that the experimental treatment considerably reduced but did not remove all residual water from $CaSO_4$-dried cysts, the average amount remaining being about 0.0069 g/g. No significant difference in cyst viability resulted from the treatment.

This estimate for residual water should be considered a maximum in terms of the cellular component of the cysts for the following reasons. First, the outer surface of the shell is very hygroscopic and it is extremely difficult to prevent the rapid uptake of very small amounts of water vapor during transfer of the cysts from the sealed tube to the balance for reference-weighing before heat-treatment began. That this uptake during transfer actually occurred is indicated by the consistent increase in the values obtained for the sequential samples of treated cysts. Thus, it is clear that at least some of the residual water of the cysts must be located in the shells, rather than the cellular component. Second, it is not unlikely that at least some of the weight loss due to heating could result from volatilization of small amounts of organic compounds from the cysts. In this regard it can be pointed out that the same treatment used on *Artemia* cysts in the present work has previously been reported to remove all detectable water from intact kangaroo tendon which binds water tenaciously[16] but does not contain the organic diversity present in intact cells. In view of these considerations and the data given here, we conclude that these cells can remain viable with residual water contents significantly lower than 0.0069 g/g.

It is worth inquiring into what such low water contents actually mean. Suppose we take the upper limit for our estimates of cyst residual water as the amount actually present in the cells after the gas bombardment treatments. The following calculations suggest that this amount of water is negligible in terms of cellular hydration requirements. Thus, the protein content of *Artemia* cysts is close to 48% of the dried weight[11]. Using the value commonly found for protein hydration (0.3 gH_2O/g dried protein) it follows that the protein present in the cysts would require about 0.144 g of water. Consequently, a residual water content of 0.0069 g/g can provide only about 5% of the amount of water needed for protein hydration alone.

Perhaps the most remarkable result of this study is that the cells somehow managed to withstand exposure to repeated cycles of the gas bombardment regime without apparent damage. The issue of residual water aside, such a result further testifies to the extraordinary ability of these cells to survive environmental insults[13].

Effect of ethylene glycol on transcription of *Neurospora crassa* conidial genome[1]

R.K. Chaudhuri

Department of Botany, Univeristy of Calcutta, 35, Ballygunge Circular Road, Calcutta-700019 (India), 7 October 1977

Summary. Ethylene glycol (EG) was found not to alter DNA sequences in *Neurospora crassa* conidia, though it is believed to be mutagenic in nature. Molecular hybridization revealed 20% increase in whole RNA transcripts in EG-treated conidia, which indicates that while untreated conidia increase RNA synthesis by 2.35fold, treated conidia are inhibited and undergo only a 1.2fold increase. Thus there is an inhibition in potential RNA synthesis, though some RNA synthesis goes on in presence of ethylene glycol.

Mutagenic action of ethylene glycol (EG) was reported in rice plants[2,3] and it was noticed that germination was greatly reduced after EG treatment[3]. Polyethylene glycol (PEG) is now extensively used in protoplast isolation without any side effect[4]. After EG treatment, conidial germination was found to be arrested[5]. But treated conidia did not lose their vitality and 75% of these were found to germinate, within 4 h, after 7 days with 18% EG treatment (unpublished data). Increase in conidial weight, volume, nuclear, mitochondrial and ribosomal numbers were reported by earlier workers[5,6]. These observations suggest that EG does not inhibit synthesis of essential macromolecules. Present work reports, that with 18% EG treatment, base sequences of *Neurospora crassa* conidial genome do not alter significantly. Also it reports slight increase (20%) in its whole RNA transcription after EG treatment.

Materials and methods. Neurospora crassa (FGSC No. 74A) conidia, 4 days old, were used for the present investigation. Incubation of conidia was maintained at 30 °C with constant agitation in 18% (v/v) EG solution with Fries' minimal medium for different periods. In control experiments conidia were found to germinate within 4 h in minimal medium. Conidia and germinating mycelia were collected, washed repeatedly with TNE buffer, pH 7.8, and stored, if

Treatment (h)	True DNA-RNA hybrid*		True DNA-DNA hybrid**		Relative HAP adsorption DNA-RNA hybrid	
	Without EG treatment	With EG treatment	Without EG treatment	With EG treatment	Without EG treatment	With EG treatment
0	14.00	13.90	92.80	92.00	1.00	1.00
4	25.90	14.00	93.00	-	1.85	1.01
8	29.00	14.20	-	92.60	2.07	1.02
24	33.00	16.50	93.20	92.50	2.35	1.19
48	32.80	16.70	93.50	92.00	2.35	1.20

*^{32}P DNA concentration: 2 µg/ml^{-1}; RNA concentration: 4 mg ml^{-1}. **^{32}P DNA concentration: 2 µg/m^{-1}; unlabelled DNA concentration: 0.5 mg ml^{-1}; incubation mixture: 0.4 M phosphate buffer, pH 6.8+0.6 M NaCl+0.01 M EDTA+50% formamide; h of incubation: 220 (maximum); temperature: 37 °C.

necessary, at −20 °C. DNA and whole RNA (w-RNA) isolation procedure, method for DNA labelling and hybridization procedures were mentioned elsewhere[7].

Results and discussion. Transcription pattern of normal and treated conidia revealed negligible amount of self (DNA-DNA) reassociation (0.5–1.0%) when w-RNA or unlabelled DNA was present much in excess of ^{32}P DNA. In different DNA-RNA reactions, ^{32}P DNA C_0t (C_0: moles ml^{-1}, t: time of reassociation) varied from 1.0×10^{-3} to 1.2×10^{-3} and R_0t (R_0: RNA moles ml^{-1}, t: time) varied from 8.5×10^4 to 8.6×10^4. Results indicate that in nongerminating normal conidia true DNA-RNA hybrid, as analyzed by hydroxyapatite chromatography, represents 14.0% of total mycelial genome. Dutta and Chaudhuri[8] reported that conidial and mycelial genome are identical in base composition. In germinating conidia transcription percent went up gradually and reached a value of 33%, i.e., a relative increase of 2.35 of *N. crassa* genome within 24 h. After EG treatment, mycelial development was found to be arrested, as expected, and w-RNA transcripts were found to increase slightly, and w-RNA transcripts were found to increase slightly, a relative increase of 1.20, which is 20% increase of 0 h transcripts. This signifies that after EG treatments some macromolecular synthesis takes place. To ascertain whether EG affects genome structure, DNA-DNA hybridizations were carried out with treated and untreated DNAs, and it was noticed that EG did not alter DNA base sequences significantly. About 92–93% of *N. crassa* genome was found to react with unlabelled DNAs from both the sources. Reassociation experiments were run upto saturation level. From the above data it appears that EG suppresses the usual RNA production in *N. crassa* conidia, though DNA structure is perhaps not affected. Slight increase in w-RNA production (about 20% or original level) in treated conidia may be due to ribosomal RNA production, and this perhaps explains the original observation of Bates and Wilson[5] that nuclear size, nuclear number and ribosome production increased after EG treatments.

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Relationship between regeneration of cell surface glycoproteins in trypsin-treated chick embryo fibroblasts and cell adhesion to the substratum

M. Vernay, M. Cornic, M. Aubery and R. Bourrillon

Centre de Recherches sur les Protéines. Faculté de Médecine, 45 rue des Saints Pères, F-75006 Paris (France), 15 November 1977

Summary The ability of cells to adhere to a substratum was altered by treatment with trypsin but was restored after a 1.5-h culture. A concomitant incorporation of [^{3}H] leucine and [^{14}C] glucosamine in the trypsin-sensitive cell surface glycoproteins was observed and almost reached a plateau within 1.50 h following the treatment with trypsin.

Cell surface glycoproteins are involved in the control of cell growth, cell differentiation and cell adhesivity[2,3]. The study of cell surface glycoproteins has been carried out following their release by trypsin and has already been well documented[4]. The present report describes the consequences of the treatment with trypsin of 8-day chick embryo fibroblasts on cell adhesion to the substratum, cell morphology in relation to the regeneration of cell surface glycoproteins.

Material and methods. Cells: 8-Day chick embryo fibroblasts were obtained as previously described[5]. Cell cultures: Cells were cultured in Falcon flasks in Eagle's medium (MEM) containing 10% foetal calf serum, 5% glutamine and 5% antibiotics. Cultures were grown in humidified air with 5% CO_2 at 37 °C. The initial seeding concentration was 10^6 cells/ml. After 48 h, the cultures reached subconfluency and they were used for the following experiments. Treatment of cells with trypsin: Cultures were washed 3 times with phosphate buffer saline (PBS) (pH 7.4) and then treated with trypsin (TPCK; 50 µg/ml; 25 °C). After 10 min of treatment, the effect of trypsin was blocked using the soybean trypsin inhibitor (100 µg/ml). Determination of cell adhesion to the substratum: After the treatment of cells with trypsin, the cells were seeded in 50 mm petri dishes in 3 ml of MEM (10^6 cells/ml) and the number of adhered cells was determined using a hemocytometer after the cells had been harvested from tissue culture dishes by trypsin. Cell viability was greater than 95% as estimated by trypan blue exclusion. The morphology of adhered cells was observed in tissue culture dishes by microscopic analyses using an inverted microscope. Incorporation of [^{3}H] leucine and [^{14}C] glucosamine: After the treatment of cells with trypsin, the cells were seeded in 50 mm petri dishes in 3 ml of MEM (10^6 cells/ml) containing 1 µCi/ml of [^{3}H] leucine (sp. act. 55 Ci/mmole, Radiochemical Centre Amersham) and 0.85 µCi/ml of [^{14}C] glucosamine (sp. act. 52 mCi/mmole, Radiochemical Centre Amersham). At various intervals, the cells were washed 3 times with PBS (pH 7.4) and were again treated with trypsin (50 µg/ml). The radioactivity incorporated with the material released by trypsin-treatment and into the trypsin-treated cells was conted in 10 ml of PCS (Amersham). The cellular protein content was estimated by the method of Lowry et al.[6].

The adhesion of cells to the substratum and the morphology of 8-day chick embryo fibroblasts following trypsin treatment

Time in h	Number of attached cells* × 10^6/dish		Cell morphology
0.5	1.2	(40%)	Most cells were spherical, a few were elongated
1	1.2	(40%)	Increase in number of cells which were elongated
1.5	2	(66%)	Fibroblastic morphology began to be restored
3	2	(66%)	Fibroblastic morphology began to be restored
4	2.2	(73%)	Complete morphology was restored to all cells

* The initial seeding concentration was 3×10^6 cells/dish. Values given in parentheses represent the percentage of attached cells as compared to the initial seeding concentration.